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(54) Title: TARGETABLE VECTOR PARTICLES

(57) Abstract

A vector particle (e.g., a retroviral vector particle) containing a chimeric envelope includes a receptor binding region binds to a receptor of a target cell. The receptor of the target cell is other than the amphotropic cell receptor. The receptor bin region may be a receptor binding region of a human virus. A portion of the envelope gene may be deleted and the deleted por is replaced with another receptor binding region or ligand. Such vector particles are targetable to a desired target cell or tis and may be administered directly to the desired target cell or tissue as part of a gene therapy procedure, or administered dire into the patient.

TARGETABLE VECTOR PARTICLES

This invention relates to "targetable" vector particles. More particularly, this invention relates to vector particles which include a receptor binding region that binds to a receptc of a target cell of a human or non-human animal.

Vector particles are useful agents for introducing gene(s) or DNA (RNA) into a cell, such as a eukaryotic cell. The gene(is controlled by an appropriate promoter. Examples of vectors which may be employed to generate vector particles include prokaryotic vectors, such as bacterial vectors; eukaryotic vectors, including fungal vectors such as yeast vectors; and viral vectors such as DNA virus vectors, RNA virus vectors, and retroviral vectors. Retroviral vectors which have been employe for generating vector particles for introducing genes or DNA (RNA) into a cell include Moloney Murine Leukemia Virus, spleen necrosis virus, and vectors derived from retroviruses such as Rous Sarcoma Virus and Harvey Sarcoma Virus. The term "introducing" as used herein encompasses a variety of methods o transferring genes or DNA (RNA) into a cell, such methods including transformation, transduction, transfection, and infection.

Vector particles have been used for introducing DNA (RNA) into cells for gene therapy purposes. In general, such a procedure involves obtaining cells from a patient and using a vector particle to introduce desired DNA (RNA) into the cells a

The envelope of murine leukemia viruses includes a protein known as gp70. Such viruses can be made "targetable" to a specific type of cell if a portion of the gp70 protein is deleted and replaced with a receptor binding region or a ligand which binds to a receptor of a target cell. Thus, in a preferred embodiment, there is provided a retroviral vector wherein a portion, but not all, of the DNA (RNA) encoding gp70 protein has been deleted and replaced with DNA (RNA) encoding a receptor binding region or a ligand which binds to a receptor of a target cell.

In general, gp70 protein includes the following regions: (i) the secretory signal or "leader" sequence; (ii) the receptor binding domain; (iii) the hinge region; and (iv) the body portion. Preferably, at least a portion of the DNA (RNA) encoding the receptor binding domain of gp70 protein is deleted and replaced with DNA (RNA) encoding a receptor binding region or a ligand which binds to a receptor of a target cell. More preferably, DNA (RNA) encoding the entire receptor binding domain of gp70 protein is deleted and replaced with DNA (RNA) encoding a receptor binding region or a ligand which binds to a receptor of a target cell. In another embodiment, DNA (RNA) encoding the entire receptor binding domain of gp70 protein, plus all or a portion of the DNA (RNA) encoding the hinge region of gp70 protein is deleted and replaced with DNA (RNA) encoding a receptor binding region or a ligand of a target cell.

The gp70 protein may be derived from an ecotropic murine leukemia virus, a xenotropic murine leukemia virus, or an amphotropic murine leukemia virus. Ecotropic gp70 (or eco gp70) (SEQ ID NO:1) is a protein having 469 amino acids, and is encoded by (SEQ ID:2). Amino acid residues 1-33 constitute the leader sequence; amino acid residues 34-263 constitute the receptor binding domain; amino acid residues 264-312 constitute the hinge region; and amino acid residues 313-469 constitute the body portion. Preferably, DNA (RNA) encoding at least a portion of

as orosomucoid, and asialofetuin. AGP is a natural high-affin ligand for ASG-R. The asialoglycoprotein receptor, or ASG-R expressed only by hepatocytes. The receptor is present at abo $3x10^5$ copies per cell, and such receptors have a high affinity for asialoglycoproteins such as AGP. Thus, the engineering of retroviral vector particles to contain asialoglycoprotein in place of the natural receptor binding domain of gp70 generates retroviral vector particles which bind to the asialoglycoprotei receptor of hepatocytes, which provides for an efficient means transferring genes of interest to liver cells.

Cell lines which generate retroviral vector particles that are capable of targeting the hepatocyte's asialoglycoprotein receptor without the removal of the particle's terminal sialic acid groups by neuraminidase treatment, can be developed by selection with the cytotoxic lectin, wheat germ agglutinin (WGA Cell lines which express the retroviral proteins gag and pol become retroviral vector packaging cell lines after they are transfected with the plasmids encoding chimeric envelope genes. These cell lines express the corresponding chimeric gp 70 glycoproteins. Upon exposure to successively higher concentrations of WGA, the outgrowth of cells which synthesize glycoproteins that lack terminal sialic acid groups, is favored (Stanley, et al., Somatic Cell Genetics, Vol. 3, pgs. 391-405 (1977)). This selection permits the isolation of cells which synthesize oligosaccharides terminating in galactosyl sugar groups. Such cells will allow the construction of packaging ce lines that are capable of generating retroviral vector particle which target the asialoglycoprotein receptor. It is also possible to select subpopulations of packaging cells which have other distinct glycotypes, such cells yielding viral vectors th potentially are capable of targeting cells other than hepatocytes. Macrophages, for example, express unique, high-mannose receptors. The PHA-resistant subpopulation will have N-linked oligosaccharides which terminate in high-mannose

mammalian genome. Another measure of rarity or scarcity of a restriction enzyme site in mammals is its representation in mammalian viruses, such as SV40. In general, an enzyme whose recognition sequence is absent in SV40 may be a candidate for being a "rare" mammalian cutter.

Examples of restriction enzyme sites having an average frequency of appearance in eukaryotic genes of less than once i 10,000 base pairs include, but are not limited to the NotI, SnaBI, SalI, XhoI, ClaI, SacI, EagI, and SmaI sites. Preferred cloning sites are selected from the group consisting of NotI, SnaBI, SalI, and XhoI.

Preferably, the multiple cloning site has a length no greater than about 70 base pairs, and preferably no greater tha about 60 base pairs. In general, the multiple restriction enzy site, or multiple cloning site is located between the 5' LTR an 3' LTR of the retroviral vector. The 5' end of the multiple cloning site is no greater than about 895 base pairs from the 3 end of the 5' LTR, preferably at least about 375 base pairs fro the 3' end of the 5' LTR. The 3' end of the multiple cloning site is no greater than about 40 base pairs from the 5' end of the 3' LTR, and preferably at least 11 base pairs from the 5' e of the 3' LTR.

Such vectors may be engineered from existing retroviral vectors through genetic engineering techniques known in the art such that the retroviral vector includes at least four cloning sites wherein at least two of the cloning sites are selected fr the group consisting of the NotI, SnaBI, SalI, and XhoI cloning sites. In a preferred embodiment, the retroviral vector includ each of the NotI, SnaBI, SalI, and XhoI cloning sites.

Such a retroviral vector may serve as part of a cloning system for the transfer of genes to such retroviral vector. Thus, there may be provided a cloning system for the manipulatic of genes in a retroviral vector which includes a retroviral vector including a multiple cloning site of the type hereinabov

targetable, whereby the receptor binding region enables the vector particles to bind to a target cell. The retroviral vec particles thus may be directly administered to a desired targe cell ex vivo, and such cells may then be administered to a patient as part of a gene therapy procedure.

Although the vector particles may be administered direct to a target cell, the vector particles may be engineered such that the vector particles are "injectable" as well as targetab i.e., the vector particles are resistant to inactivation by hur serum, and thus the targetable vector particles may be administered to a patient by intravenous injection, and travel directly to a desired target cell or tissue without being inactivated by human serum.

The envelope of retroviruses also includes a protein know as p15E, and Applicants have found that retroviruses are susceptible to inactivation by human serum a a result of the action of complement protein(s) present in serum on the p15E protein portion of the retrovirus. Applicants have further fou that such retroviruses can be made resistant to inactivation by human serum by mutating such p15E protein.

In one embodiment, therefore, the retroviral vector is engineered such that a portion of the DNA (RNA) encoding p15E protein (shown in the accompanying sequence listing as SEQ ID NO:7), has been mutated to render the vector particle resistant to inactivation by human serum; i.e., at least one amino acid b not all of the amino acids of the p15E protein has been changed or mutated.

p15E protein is a viral protein having 196 amino acid residues. In viruses, sometimes all 196 amino acid residues ar present, and in other viruses, amino acid residues 181 to 196 (known as the "r" peptide), are not present, and the resulting protein is the "mature" form of p15E known as p12E. Thus, viruses can contain both the p15E and p12E proteins. p15E protein is anchored in the viral membrane such that amino acid

In one embodiment, the mutation of DNA (RNA) encoding p15 protein may be effected by deleting a portion of the p15E gene and replacing the deleted portion of the p15E gene with fragment(s) or portion(s) of a gene encoding another viral protein. In one embodiment, one portion of DNA encoding the p protein is replaced with a fragment of the gene encoding the p protein, which is an HTLV-I transmembrane protein. HTLV-I vir has been found to be resistant to binding by complement protei and thus HTLV-I is resistant to inactivation by human serum (Hoshino, et al., Nature, Vol. 310, pgs. 324-325 (1984)). Thus in one embodiment, there is also provided a retroviral vector particle wherein a portion of the p15E protein has been deletec and replaced with a portion of another viral protein, such as a portion of the p21 protein.

p21 protein (as shown in the accompanying sequence listing as SEQ ID NO:8) is a protein having 176 amino acid residues, ar which, in relation to p15E, has significant amino acid sequence homology. In one embodiment, at least amino acid residues 39 to 61, and 101 to 123 are deleted from p15E protein, and replaced with amino acid residues 34 to 56 and 96 to 118 of p21 protein. In one alternative, at least amino acid residues 39 to 123 of p15E protein are deleted and replaced with amino acid residues to 118 of p21 protein.

In another embodiment, amino acid residues 39 to 69 of p15 protein are deleted and replaced with amino acid residues 34 to 64 of p21 protein, and amino acid residues 96 to 123 of p15E protein are deleted and replaced with amino acid residues 91 to 118 of p21 protein.

Vector particles generated from such packaging lines, therefore, are "targetable" and "injectable," whereby such vecto particles, upon administration to a patient, travel directly to desired target cell or tissue.

The targetable vector particles are useful for the introduction of desired heterologous genes into target cells ex

Simplex virus particles), or synthetic particles may be constructed such that the vector particles include a receptor binding region that binds to a receptor of a target cell, wher the receptor of a human target cell is other than the amphotro cell receptor. Such vector particles are suitable for in vivo administration to a desired target cell.

Advantages of the present invention include the ability t provide vector particles which may be administered directly to desired target cell or tissues, whereby desired genes are delivered to the target cell or tissue, whereby the target cell or tissue may produce the proteins expressed by such genes.

This invention will now be described with respect to the following examples; however, the scope of the present invention is not intended to be limited thereby.

Example 1

Plasmid pCee (Figure 1), which contains the ecotropic muri leukemia virus gp70 and p15E genes under the control of a CMV promoter, was cut with AccI, and an AccI fragment encoding amin acid residues 1-312 of the eco gp70 protein was removed. Clone into the AccI site was a PCR fragment containing the eco gp70 secretion signal (or leader, which includes amino acid residues 1-33 of eco gp70), followed by mature rabbit alpha-1 acid glycoprotein (amino acid residues 19-201) (Ray, et al., Biochemical and Biophysical Research Communications, Vol. 178, No. 2, pgs. 507-513 (1991)). The amino acid sequence of rabbit alpha-1 acid glycoprotein is shown in (SEQ ID NO:5), and the DN sequence encoding therefor is shown in (SEQ ID NO:6). The resulting plasmid pAGP-1 (Figure 2) contains the eco gp70 leade sequence (amino acid residues 1-33 of eco gp70), a sequence encoding the mature rabbit alpha-1 acid glycoprotein (amino aci residues 19-201), and a sequence encoding amino acid residues 3 to 469 of eco gp70.

Example 2

Example 3

Plasmid pUC18RSVXeno (Figure 4), which contains the xenotrophic murine leukemia virus gp70 and p15E genes under t control of an RSV promoter, was cut with AccI and StuI, and a AccI-StuI fragment encoding amino acid residues 1-258 of xenc gp70 was removed. Cloned into this site was a PCR generated AccI-StuI fragment encoding the xeno gp70 leader (amino acid residues 1-30), and the mature rabbit alpha-1 acid glycoprote The resulting plasmid, pAX2 (Figure 5), thus contains a sequer encoding the xeno gp70 leader, a sequence encoding the mature rabbit alpha-1 acid glycoprotein, and amino acid residues 259- of xeno gp70.

Example 4

Plasmid pUC18RSVXeno was cut with AccI and ClaI, and a fragment encoding amino acid residues 1-210 of xeno gp70 was removed. Cloned into this site was a PCR generated AccI-ClaI fragment encoding the xeno gp70 leader, followed by mature rab alpha-1 acid glycoprotein. The resulting plasmid, pAX6 (Figur 6), thus includes a sequence encoding the xeno gp70 leader, a sequence encoding mature rabbit alpha-1 acid glycoprotein, and amino acid residues 211-443 of xeno gp70.

Example 5

$5x10^5$ GPL cells on 10 cm tissue culture plates were transfected (using $CaPO_4$) with 30 µg/plate of one of plasmid pAGP-1, pAGP-3, pAX2, or pAX6. The $CaPO_4$ is removed 24 hours later and 10 ml of fresh D10 medium is added for another 24 hours. The D10 medium is then removed and replaced with serum free DX medium for another 24 hours. The DX medium is then collected, filtered, and stored on ice. This supernatant contains the vector particles.

The supernatants were then filtered and collected by standard procedures and then centrifuged. After centrifugatio the virus pellets were reconstituted in a buffer containing 0. sodium acetate, 0.15M sodium chloride, and 2mM calcium chlorid

were plated, 1 ml of D10 was removed from the first well and 2 of neuraminidase-treated (or untreated as a control) viral supernatant containing Chimeric-1 or Chimeric-3 was added and mixed well. 200 ul from the first well was diluted into the 2 present in the second well, was then mixed; and then 200 ul fro the second well was diluted into the 1.8 ml present in the thir well, thereby giving approximate dilutions of 2/3, 1/15, and 1/150. 8 ug/ml of Polybrene was included in each well during t transduction. The viral particles were left in contact with th cells overnight, followed by removal of media containing viral particles, and replaced with D10 containing 1,000 mg/ml of G418 The medium was changed with fresh D10 and G418 every 4 to 5 day as necessary. G418-resistant colonies were scored after 2 to 3 weeks.

Example 6

The pre-packaging cell line GP8, which expresses the retroviral proteins gag and pol, and the packaging cell lines derived from them which also express the chimeric gp70 glycoproteins encoded by the plasmids pAGP-1, pAGP-3, pAX2, or pAX6 were maintained in cell culture and exposed to successively higher concentrations of wheat germ agglutinin; starting with 1 ug/ml. The cell lines were maintained under WGA selection in cell culture for 6 to 8 weeks until populations resistant to 40-50 ug/ml WGA were obtained. The latter were then subjected t fluoresence-activated cell sorting using FITC-conjugated lectins to enrich for the cells expressing the desired mutant glycotype (e.g., FITC-Erythrina Cristagalli agglutinin for beta-D-galactosyl groups, and FITC-concanavalin A for alpha-D-mannosyl groups). Retroviral vector packaging and producer cell lines were then generated from the resulting populations by standard techniques.

It is to be understood, however, that the scope of the present invention is not to be limited to the specific embodiments described above. The invention may be practiced

PATAP697

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Anderson, W. French
Baltrucki, Leon F.
Mason, James M.

(ii) TITLE OF INVENTION: Targetable Vector Particles

(iii) NUMBER OF SEQUENCES: 8

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Carella, Byrne, Bain, Gilfilla
Cecchi & Stewart
(B) STREET: 6 Becker Farm Road
(C) CITY: Roseland
(D) STATE: New Jersey
(E) COUNTRY: USA
(F) ZIP: 07068

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: 3.5 inch diskette
(B) COMPUTER: IBM PS/2
(C) OPERATING SYSTEM: PC-DOS
(D) SOFTWARE: DW4.V2

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 469 bases

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME/KEY: Ecotropic gp70 Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Met Ala Arg Ser Thr Leu Ser Lys Pro Leu
5 10
Lys Asn Lys Val Asn Pro Arg Gly Pro Leu
15 20
Ile Pro Leu Ile Leu Leu Met Leu Arg Gly
25 30
Val Ser Thr Ala Ser Pro Gly Ser Ser Pro
35 40
His Gly Val Tyr Asn Ile Thr Trp Glu Val
45 50
Thr Asn Gly Asp Arg Glu Thr Val Trp Ala
55 60
Thr Ser Gly Asn His Pro Leu Trp Thr Trp
65 70
Trp Pro Asp Leu Thr Pro Asp Leu Cys Met
75 80
Leu Ala His His Gly Pro Ser Tyr Trp Gly
85 90
Leu Glu Tyr Gln Ser Pro Phe Ser Ser Pro
95 100
Pro Gly Pro Pro Cys Cys Ser Gly Gly Ser

275 280
Ser Lys Pro Lys Pro Val Lys Ser Pro Ser
285 290
Val Thr Lys Pro Pro Ser Gly Thr Pro Leu
295 300
Ser Pro Thr Gln Leu Pro Pro Ala Gly Thr
305 310
Glu Asn Arg Leu Leu Asn Leu Val Asp Gly
315 320
Ala Tyr Gln Ala Leu Asn Leu Thr Ser Pro
325 330
Asp Lys Thr Gln Glu Cys Trp Leu Cys Leu
335 340
Val Ala Gly Pro Pro Tyr Tyr Glu Gly Val
345 350
Ala Val Leu Gly Thr Tyr Ser Asn His Thr
355 360
Ser Ala Pro Ala Asn Cys Ser Val Ala Ser
365 370
Gln His Lys Leu Thr Leu Ser Glu Val Thr
375 380
Gly Gln Gly Leu Cys Ile Gly Ala Val Pro
385 390
Lys Thr His Gln Ala Leu Cys Asn Thr Thr
395 400
Gln Thr Ser Ser Arg Gly Ser Tyr Tyr Leu
405 410
Val Ala Pro Thr Gly Thr Met Trp Ala Cys
415 420
Ser Thr Gly Leu Thr Pro Cys Ile Ser Thr
425 430
Thr Ile Leu Asn Leu Thr Thr Asp Tyr Cys
435 440
Val Leu Val Glu Leu Trp Pro Arg Val Thr

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 1446 bases

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: viral DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GGCTGCCGAC CCCGGGGGTG GACCATCCTC TAGACTGACA TGGCGCGTTA AACGCTCTCA

AAACCCCTTA AAAATAAGGT TAACCCGCGA GGCCCCCTAA TCCCCTTAAT TCTTCTGATG

CTCAGAGGGG TCAGTACTGC TTCGCCCGGC TCCAGTCCTC ATCAAGTCTA TAATATCACC

TGGGAGGTAA CCAATGGAGA TCGGGAGACG GTATGGGCAA CTTCTGGCAA CCACCCTCTG

TGGACCTGGT GGCCTGACCT TACCCCAGAT TTATGTATGT TAGCCCACCA TGGACCATCT

TATTGGGGGC TAGAATATCA ATCCCCTTTT TCTTCTCCCC CGGGGCCCCC TTGTTGCTCA

GGGGGCAGCA GCCCAGGCTG TTCCAGAGAC TGCGAAGAAC CTTTAACCTC CCTCACCCCT

CGGTGCAACA CTGCCTGGAA CAGACTCAAG CTAGACCAGA CAACTCATAA ATCAAATGAG

GGATTTTATG TTTGCCCCGG GCCCCACCGC CCCCGAGAAT CCAAGTCATG TGGGGGTCCA

GACTCCTTCT ACTGTGCCTA TTGGGGCTGT GAGACAACCG GTAGAGCTTA CTGGAAGCCC

TCCTCATCAT GGGATTTCAT CACAGTAAAC AACAATCTCA CCTCTGACCA GGCTGTCCAG

GTATGCAAAG ATAATAAGTG GTGCAACCCC TTAGTTATTC GGTTTACAGA CGCCGGGAGA

CGGGTTACTT CCTGGACCAC AGGACATTAC TGGGGCTTAC GTTTGTATGT CTCCGGACAA

GATCCAGGGC TTACATTTGG GATCCGACTC AGATACCAAA ATCTAGGACC CCGCGTCCCA

ATAGGGCCAA ACCCCGTTCT GGCAGACCAA CAGCCACTCT CCAAGCCCAA ACCTGTTAAG

TCGCCTTCAG TCACCAAACC ACCCAGTGGG ACTCCTCTCT CCCCTACCCA ACTTCCACCG

GCGGGAACGG AAAATAGGCT GCTAAACTTA GTAGACGGAG CCTACCAAGC CCTCAACCTC

ACCAGTCCTG ACAAAACCCA AGAGTGCTGG TTGTGTCTAG TAGCGGGACC CCCCTACTAC

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 443 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME/KEY:xenotropic gp70 protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Met Glu Gly Ser Ala Phe Ser Lys Pro Leu
5 10

Lys Asp Lys Ile Asn Pro Trp Gly Pro Leu
15 20

Ile Val Met Gly Ile Leu Val Arg Ala Gly
25 30

Ala Ser Val Gln Arg Asp Ser Pro His Gln
35 40

Ile Phe Asn Val Thr Trp Arg Val Thr Asn
45 50

Leu Met Thr Gly Gln Thr Ala Asn Ala Thr
55 60

Ser Leu Leu Gly Thr Met Thr Asp Thr Phe
65 70

Phe Thr Asp Ala Gly Arg Lys Ala Ser Trp
195 200

Asp Ala Pro Lys Val Trp Gly Leu Arg Leu
205 210

Tyr Arg Ser Thr Gly Ala Asp Pro Val Thr
215 220

Arg Phe Ser Leu Thr Arg Gln Val Leu Asn
225 230

Val Gly Pro Arg Val Pro Ile Gly Pro Asn
235 240

Pro Val Ile Thr Asp Gln Leu Pro Pro Ser
245 250

Gln Pro Val Gln Ile Met Leu Pro Arg Pro
255 260

Pro His Pro Pro Pro Ser Gly Thr Val Ser
265 270

Met Val Pro Gly Ala Pro Pro Pro Ser Gln
275 280

Gln Pro Gly Thr Gly Asp Arg Leu Leu Asn
285 290

Leu Val Glu Gly Ala Tyr Gln Ala Leu Asn
295 300

Leu Thr Ser Pro Asp Lys Thr Gln Glu Cys
305 310

Trp Leu Cys Leu Val Ser Gly Pro Pro Tyr
315 320

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 1356 bases

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: viral DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GCGACAACTC CTCCAGCCGG GAACAGCATG GAAGGTTCAG CGTTCTCAAA ACCCCTTAAA

GATAAGATTA ACCCGTGGGG CCCCCTAATA GTTATGGGGA TCTTGGTGAG GGCAGGAGCT

TCGGTACAAC GTGACAGCCC TCACCAGATC TTCAATGTTA CTTGGAGAGT TACCAACCTA

ATGACAGGAC AAACAGCTAA CGCCACCTCC CTCCTGGGGA CGATGACAGA CACCTTCCCT

AAACTATATT TTGACCTGTG TGATTTAGTA GGAGACTACT GGGATGACCC AGAACCCGAT

ATTGGGGATG GTTGCCGCAC TCCCGGGGGA AGAAGAAGGA CAAGACTGTA TGACTTCTAT

GTTTGCCCCG GTCATACTGT ACCAATAGGG TGTGGAGGGC CGGGAGAGGG CTACTGTGGC

AAATGGGGAT GTGAGACCAC TGGACAGGCA TACTGGAAGC CATCATCATC ATGGGACCTA

ATTTCCCTTA AGCGAGGAAA CACTCCTAAG GATCAGGGCC CCTGTTATGA TTCCTCGGTC

TCCAGTGGCG TCCAGGGTGC CACACCGGGG GGTCGATGCA ACCCCCTGGT CTTAGAATTC

ACTGACGCGG GTAGAAAGGC CAGCTGGGAT GCCCCCAAAG TTTGGGGACT AAGACTCTAT

CGATCCACAG GGGCCGACCC GGTGACCCGG TTCTCTTTGA CCCGCCAGGT CCTCAATGTA

GGACCCCGCG TCCCCATTGG GCCTAATCCC GTGATCACTG ACCAGCTACC CCCATCCCAA

CCCGTGCAGA TCATGCTCCC CAGGCCTCCT CATCCTCCTC CTTCAGGCAC GGTCTCTATG

GTACCTGGGG CTCCCCCGCC TTCTCAACAA CCTGGGACGG GAGACAGGCT GCTAAATCTG

GTAGAAGGAG CCTACCAAGC ACTCAACCTC ACCAGTCCTG ACAAAACCCA AGAGTGCTGG

TTGTGTCTGG TATCGGGACC CCCCTACTAC GAAGGGCTTG CCGTCCTAGG TACCTACTCC

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 201 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE

(A) NAME/KEY: rabbit alpha-1-acid glycoprotein

(x) PUBLICATION INFORMATION

(A) AUTHOR Ray, et al.

(B) TITLE:

(C) JOURNAL: Biochem. and Biophys. Res. Comm.

(D) VOLUME: 178

(E) ISSUE: No. 2

(F) PAGES: 507-513

(G) DATE: 1991

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

```
Met Ala Leu Pro Trp  Ala Leu Ala Val Leu
                 5                  10
Ser Leu Leu Pro Leu  Leu His Ala Gln Asp
                15                  20
Pro Ala Cys Ala Asn  Phe Ser Thr Ser Pro
                25                  30
```

Glu Ala Leu Thr Cys Leu Gly Met Asn Lys
165 170

Thr Glu Val Val Tyr Thr Asp Trp Thr Lys
175 180

Asp Leu Cys Glu Pro Leu Glu Lys Gln His
185 190

Glu Glu Glu Arg Lys Lys Glu Lys Ala Glu
195 200

Ser

ACAAGACGGA AGTCGTCTAC ACTGACTGGA CAAAGGATCT GTGCGAGCCG CTGGAGAAGC

AACACGAGGA GGAGAGGAAG AAGGAAAAGG CAGAGTCATA GGGCACAGCA CCGGCTCCGG

GACTCGGGGC CCACCCCCTG CACCTGCCTT TTTGTTTGTT TTGTAAATCT CTGTTCTTTC

CCATGGTTGC ATCAATAAAA CTGCTGGACC AGTAAAAAA

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 196 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME/KEY: ecotropic p15E protein.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Glu Pro Val Ser Leu Thr Leu Ala Leu Leu
5 10

Leu Gly Gly Leu Thr Met Gly Gly Ile Ala
15 20

Ala Gly Ile Gly Thr Gly Thr Thr Ala Leu
25 30

Met Ala Thr Gln Gln Phe Gln Gln Leu Gln
35 40

Ala Ala Val Gln Asp Asp Leu Arg Glu Val
45 50

Val Leu Thr Gln Gln Tyr His Gln Leu Lys

185 190

Pro Ile Glu Tyr Glu Pro

195

INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 176 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME/KEY: HTLV-I p21 protein

(x) PUBLICATION INFORMATION:

(A) AUTHOR: Malik, et al.

(B) TITLE:

(C) JOURNAL: J. Gen. Virol.

(D) VOLUME: 69

(E) ISSUE:

(F) PAGES: 1695-1710

(G) DATE: 1988

Lys Asn His Lys Asn Leu Leu Lys Ile Ala
55 60
Gln Tyr Ala Ala Gln Asn Arg Arg Gly Leu
65 70
Asp Leu Leu Phe Trp Glu Gln Gly Gly Leu
75 80
Cys Lys Ala Leu Gln Glu Gln Cys Cys Phe
85 90
Leu Asn Ile Thr Asn Ser His Val Ser Ile
95 100
Leu Gln Glu Arg Pro Pro Leu Glu Asn Arg
105 110
Val Leu Thr Gly Trp Gly Leu Asn Trp Asp
115 120
Leu Gly Leu Ser Gln Trp Ala Arg Glu Ala
125 130
Leu Gln Thr Gly Ile Thr Leu Val Ala Leu
135 140
Leu Leu Leu Val Ile Leu Ala Gly Pro Cys
145 150
Ile Leu Arg Gln Leu Arg His Leu Pro Ser
155 160
Arg Val Arg Tyr Pro His Tyr Ser Leu Ile
165 170
Asn Pro Glu Ser Ser Leu
175

9. The vector particle of Claim 8 wherein said protein which binds to an asialoglycoprotein receptor of hepatocytes is alpha-1 acid glycoprotein.

10. The vector particle of Claim 1 and further including at least one heterologous gene.

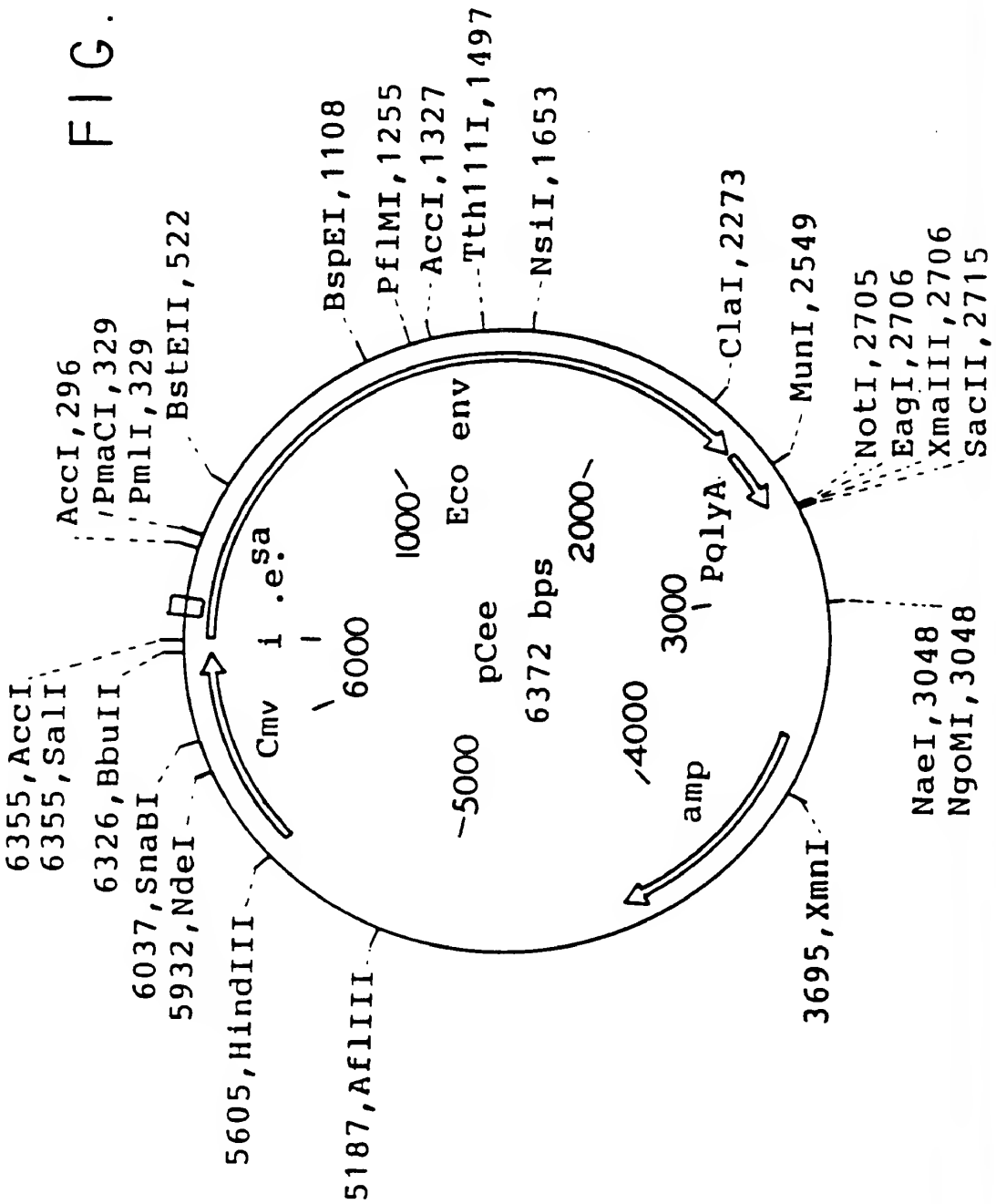
11. A method of introducing at least one heterologous gene into a target cell, comprising, administering to said target cell the vector particles of Claim 10.

12. The method of Claim 11 wherein said vector particles are administered ex vivo.

13. The method of Claim 11 wherein said vector particles are administered in vivo.

14. A packaging cell line which produces the retroviral particles of Claim 1.

FIG. 1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EagI,2706
XmaIII,2706
SacII,2715
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6355,SalI
6326,BbuII
6037,SnaBI
5932,NdeI
5605,HindIII
5187,AflIII
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pCee
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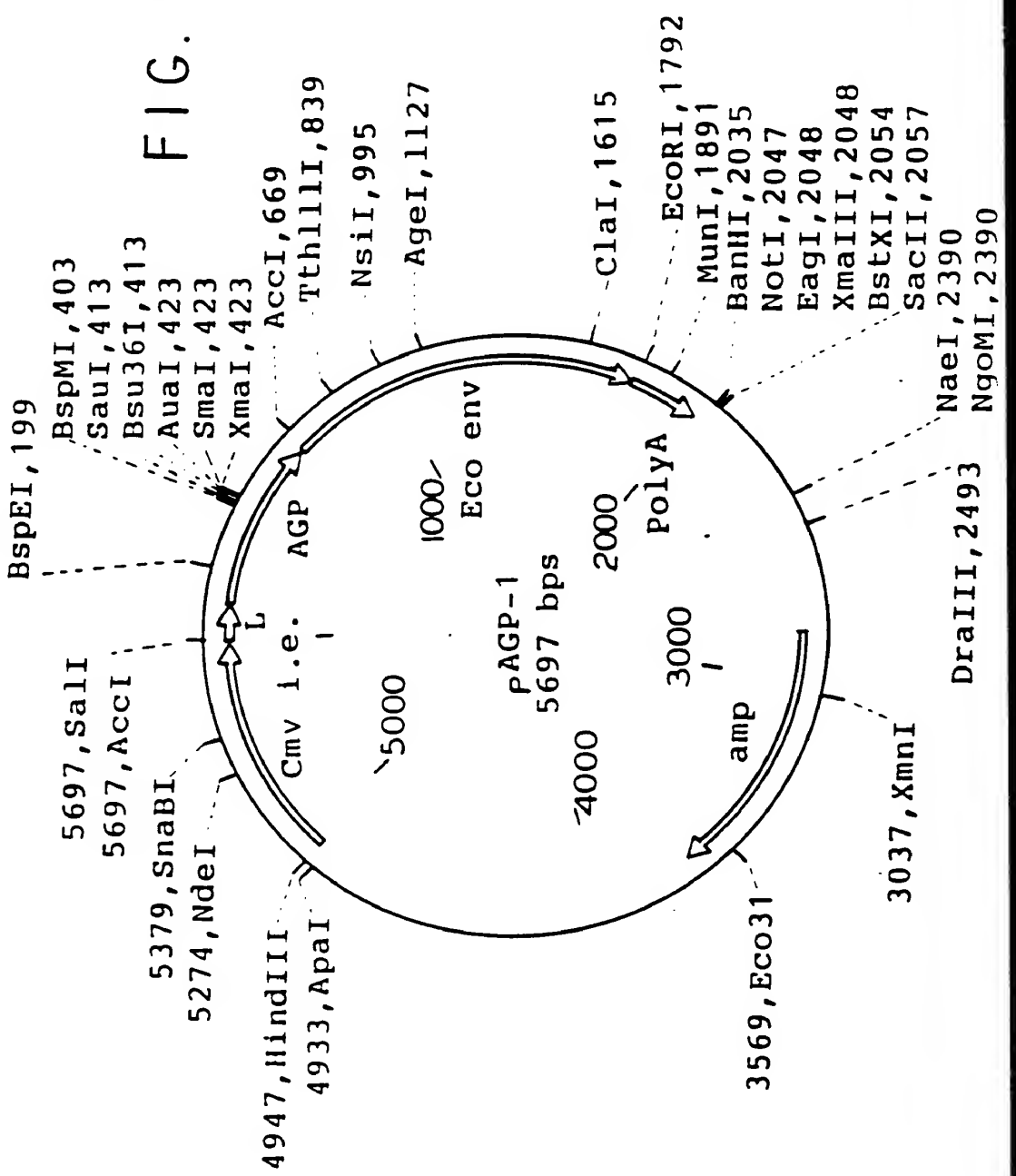
FIG. 2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XmaI,423
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NsiI,995
AgeI,1127
ClaI,1615
EcoRI,1792
MunI,1891
BanHI,2035
NotI,2047
EagI,2048
XmaIII,2048
BstXI,2054
SacII,2057
NaeI,2390
NgoMI,2390
DraIII,2493
3037,XmnI
3569,Eco31
4947,HindIII
4933,ApaI
5379,SnaBI
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amp
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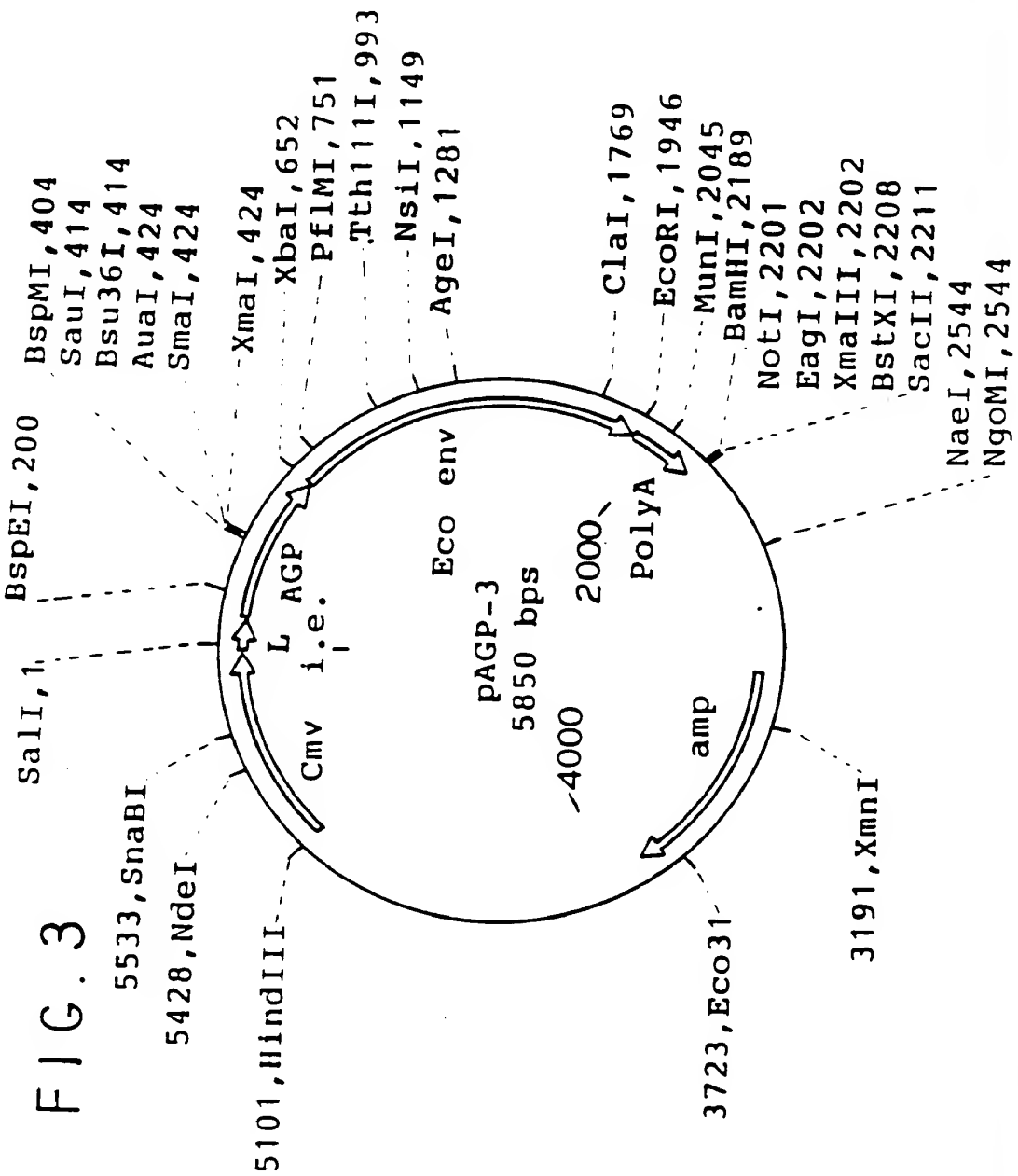
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SmaI,424
XmaI,424
XbaI,652
Pf1MI,751
Tth111I,993
NsiI,1149
AgeI,1281
ClaI,1769
EcoRI,1946
MunI,2045
BamHI,2189
NotI,2201
EagI,2202
XmaIII,2202
BstXI,2208
SacII,2211
NaeI,2544
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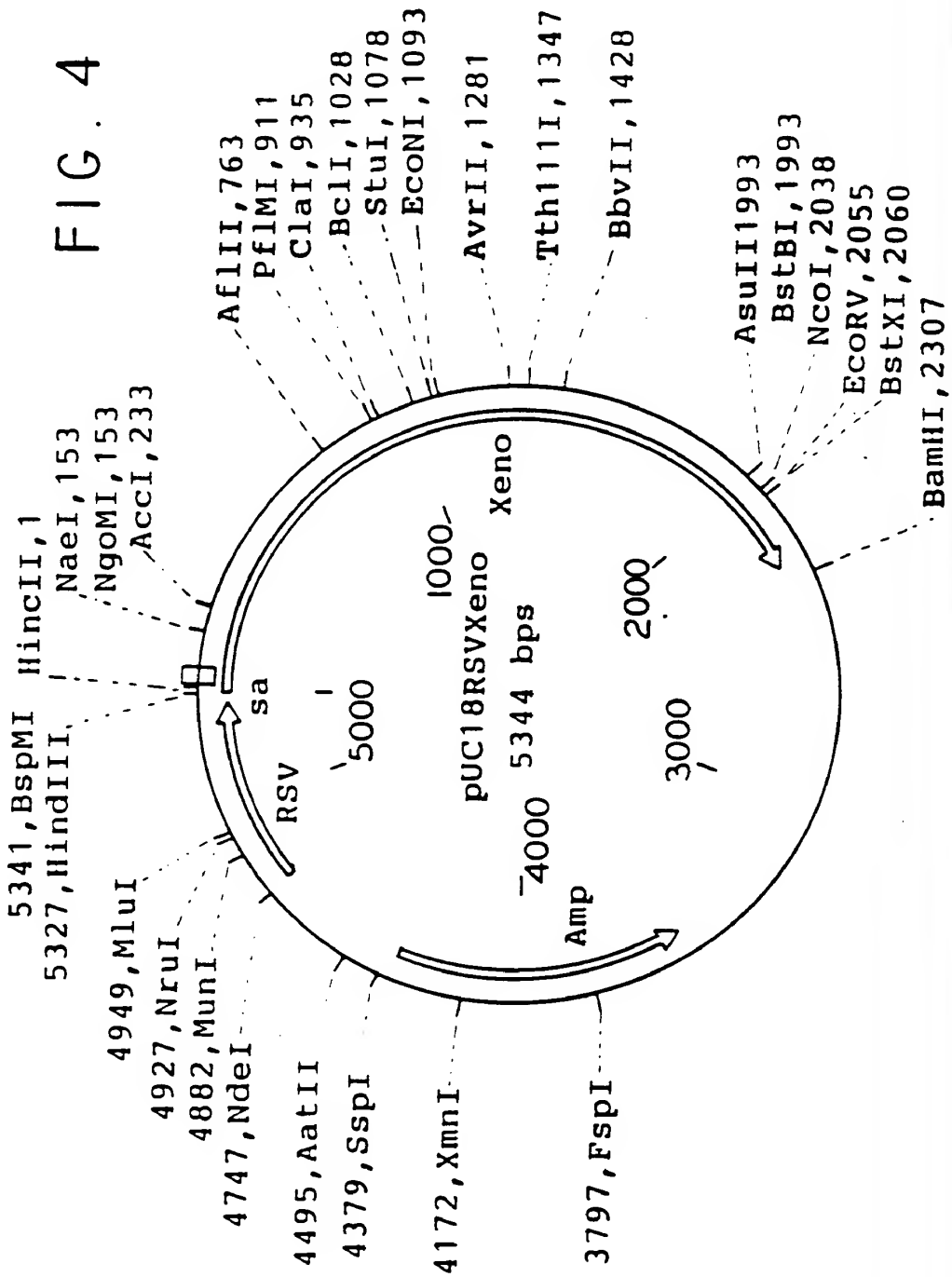
5428,NdeI
5533,SnaBI

FIG. 3

FIG. 4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BclI,1028
StuI,1078
EcoNI,1093
AvrII,1281
Tth111I,1347
BbvII,1428
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BstBI,1993
NcoI,2038
EcoRV,2055
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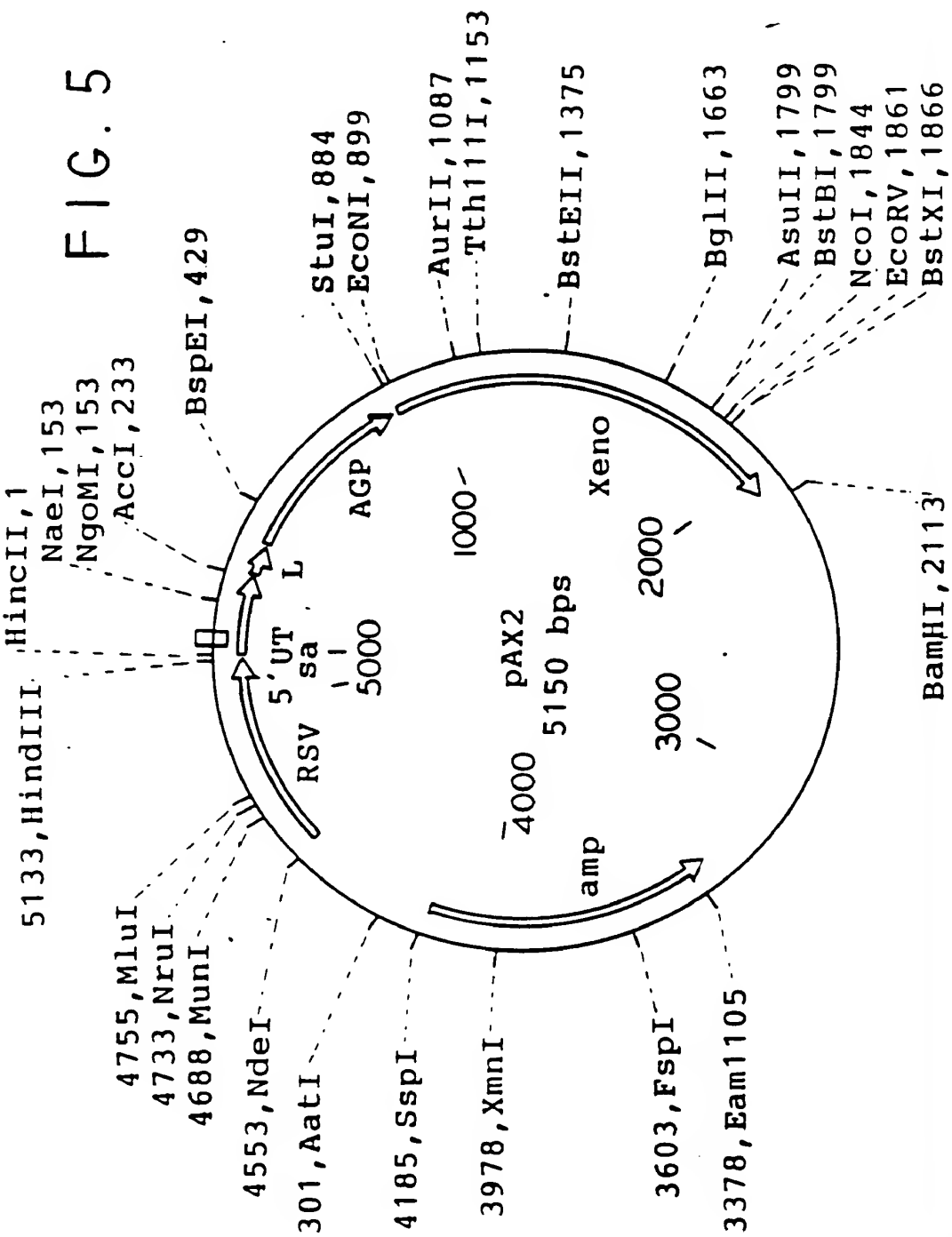
4172,XmnI
3797,FspI

FIG. 5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Tth111I,1153
BstEII,1375
BglII,1663
AsuII,1799
BstBI,1799
NcoI,1844
EcoRV,1861
BstXI,1866
BamHI,2113
4755,MluI
4733,NruI
4688,MunI
4553,NdeI
4301,AatI
4185,SspI
3978,XmnI
3603,FspI
3378,Eam1105
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amp
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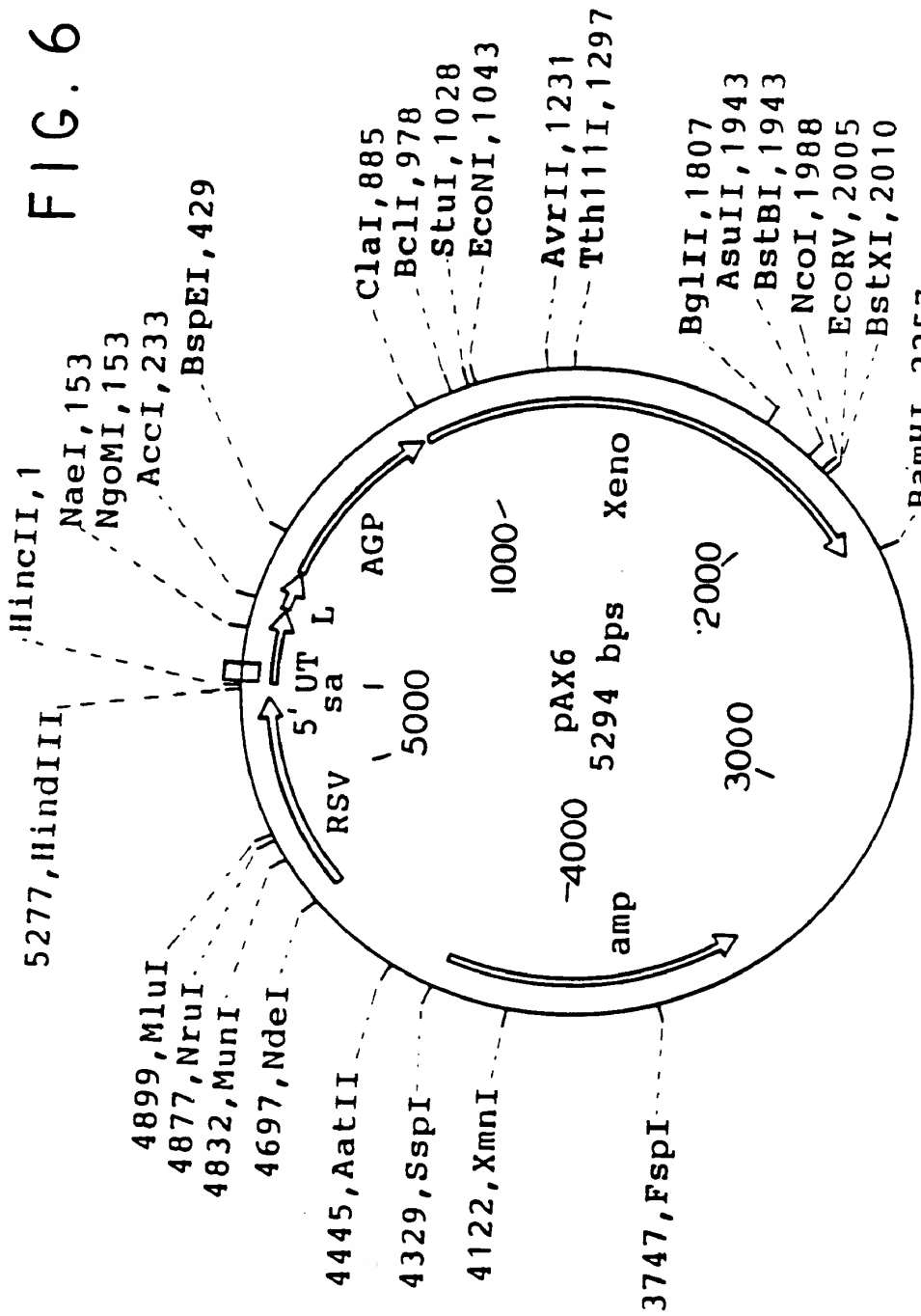
4000
5000

FIG. 6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AvrII,1231
Tth111I,1297
BglII,1807
AsuII,1943
BstBI,1943
NcoI,1988
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BstXI,2010
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5277,HindIII
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4697,NdeI
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4329,SspI
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/10522

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C12P 21/00; C12N 15/00, 15/58, 15/40, 15/48, 15/63, 15/86
US CL :435/320.1, 69.1, 240.2; 424/93; 935/23, 32, 52, 57, 66, 70
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/320.1, 69.1, 240.2; 424/93; 935/23, 32, 52, 57, 66, 70

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Dialog, Biosis, Biotech, Medicine, Medline
Search Terms: retrovirus, vector, receptor, receptor binding protein

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Journal of Virology, Volume 61, No. 5, issued May 1987, M.A. Bender et al., "Evidence that the Packaging Signal of Moloney Murine Leukemia Virus Extends Into the gag Region" pages 1639-1646, See particularly page 1640.	1-14
A	Biotechniques, Volume 7, No. 9, issued 1989, A.D. Miller et al., "Improved Retroviral Vectors for Gene Transfer and Expression" pages 980-990, See particularly page 984.	1-14

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 05 JANUARY 1994	Date of mailing of the international search report 26 JAN 1994
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer DAVID GUZO Telephone No. (703) 308-0196

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